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Evaluation of Serum hs-CRP Levels in Patients with *Helicobacter pylori* Infection Confirmed by Non-Invasive Tests

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Abstract

Background and Aim: Diagnosing *Helicobacter pylori* infection non-invasively remains a challenge, and upper endoscopy is often relied upon unnecessarily. This study aimed to assess the diagnostic accuracy of combining high-sensitivity C-reactive protein (hs-CRP) with a standard non-invasive test, the urea breath test (UBT), and to compare the results with the stool antigen (Stool Ag) test, in order to improve diagnostic accuracy and reliability and to reduce dependence on diagnostic upper endoscopy.

Methods: The study included 150 patients with dyspeptic symptoms (89 males, 59.3%; 61 females, 40.7%), with a mean age of 34.19 years. Statistical analyses were performed using IBM SPSS Statistics and included Welch's t-test, Fisher's exact test, and ROC analysis to determine diagnostic performance and optimal cut-off points. UBT was used as the validated reference standard against which hs-CRP and the Stool Ag test were compared.

Results: UBT showed a positivity rate of 98%. Mean hs-CRP was significantly higher in the positive group (67.52 ± 38.11 mg/L) than in the negative group (0.96 ± 0.81 mg/L; $p < 0.001$, Welch's t-test). On ROC analysis, hs-CRP yielded an AUC of 1.000, indicating optimal predictive performance at a threshold of >10 mg/L, with 97.28% sensitivity and 100% specificity. The kappa value of 0.588 indicated moderate agreement between the diagnostic methods used.

Conclusion: Although hs-CRP is a broad indicator of systemic inflammation rather than a pathogen-specific diagnostic test, its evaluation alongside UBT may reflect the magnitude of the associated inflammatory response in symptomatic patients.

Keywords: *Helicobacter pylori;* High-sensitivity C-reactive protein; Urea breath test; Stool antigen test.

1. Introduction

Pathogenic bacterial infections are among the most widespread diseases globally, including those affecting the digestive system, particularly *Helicobacter pylori* (*H. pylori*). Global statistics indicate that 50% of the world's population is infected with this bacterium (1). It is the leading cause of many upper gastrointestinal infections and can sometimes lead to cancer, stomach ulcers, or chronic gastritis. Early diagnosis using accurate diagnostic techniques is crucial for controlling the infection and its complications (2). Interventional methods such as biopsies or endoscopy are the first line of diagnosis in such cases, but their use is often limited by the invasive nature of the procedure and the potential for errors during sample collection (3). For this reason, other diagnostic tests, including urea breath test (UBT) and the stool antigen test (Stool Ag), have been developed due to their good accuracy in detecting the bacteria. However, the accuracy of these tests can be affected by various internal and external factors, leading to inaccurate results (4, 5). Recently, clinical diagnosis has increasingly focused on detecting systemic markers of inflammation, particularly hs-CRP testing, which has been considered a complementary tool in assessing the level of inflammation associated with this type of bacteria (6). It is known that the hs-CRP test provides an indication of subclinical inflammation in the human body; this is because it provides an indicator of systemic responses to inflammation, including inflammation in the stomach. However, its combined use with UBT and

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the stool Ag test is not yet fully understood, particularly regarding its classification as a multimodal diagnostic tool. This study investigates the synergistic predictive value between (hs-CRP) and (UBT), and fecal antigen in the diagnosis of *H. pylori* infection. The combined analysis of these data aims to determine and evaluate (hs-CRP) as an adjunctive inflammatory marker, alongside the (UBT) and the stool antigen test.

2. Methods

2.1. Location and Study Design:

This was a cross-sectional study to assess the diagnostic accuracy and predictive value of three tests (hs-CRP, UBT, and stool Ag test) in patients with dyspepsia symptoms referred for laboratory testing for the diagnosis of *H. pylori* infection. The study was carried out at Al-Hussein Teaching Hospital and Al-Amal Specialized Medical Laboratory in a specific geographical area encompassing the city of Al-Samawah, the capital of Al-Muthanna Governorate in Iraq. The study was conducted from June 2025 to March 2026. Written informed consent was obtained from all participants in the study, in accordance with the Declaration of Helsinki (7).

2.2. Patient Selection and Gold Standard.

Individuals showing signs of undiagnosed dyspepsia were selected using a simple random sampling method to avoid selection bias. The 13C-urea breath test (UBT) was utilized as the sole reference standard for diagnosing active H. pylori infection. This selection aligns with international clinical guidelines (Maastricht VI Consensus), which regard the 13C-UBT as a non-invasive gold standard due to its exceptional diagnostic sensitivity and specificity, both of which exceed 95–98%.

2.3 Research Criteria

The study included 150 patients with no upper age limit (89 males and 61 females) who presented to the hospital with symptoms of gastritis, peptic ulcer, and dyspepsia. The study participants were selected using the random selection method.

Selection Criteria

Patients aged ≥ 17 years who were confirmed not to be receiving any treatment for H. pylori infection

Exclusion Criteria

All patients who used proton pump inhibitors (PPIs) or treatment protocols that may suppress bacterial activity (8), such as bismuth subsalicylate therapy, antibiotics, or probiotics therapy during the month prior to analysis, patients who had recent surgery, and patients with chronic inflammatory diseases (such as rheumatoid arthritis) that may show elevated (hs-CRP) results unrelated to H.pylori were excluded (9).

2.4. Laboratory Diagnosis

Urea Breath Test (UBT): For this test, patients were instructed to fast for 4–6 hours prior to the test. A medically safe urea solution was used, and a second sample was collected 15 minutes later (10), This test has been made a reference test for infection. number (1) was used for a (positive) result and (0) for a (negative) result in the SPSS data analysis.

Stool antigen (Stool Ag) test: Stool samples were collected in sterile containers; Patients were advised not to use any laxatives. Analyzed using Lateral flow chromatography (LFC) Technology for the detection of H. pylori antigen in stool samples by rapid test strip from Ctk biotech made in USA (11). number (1) was used for a (positive) result and (0) for a (negative) result in the SPSS data analysis.

hs-CRP test: Blood samples were collected from patients' veins in a sterile environment and placed in gel tubes for centrifugation at 5000 rpm for 5 minutes to obtain serum. hs-CRP levels were measured (Immunofluorescence Assay Technology) by GP-Getein, model: GP-1100, the measurement range was 0.5-200 mg\L and cut-off value (positive) >10 mg\L (12).

Laboratory tests were performed by trained personnel who were unaware of the patients' clinical conditions and other tests in order to prevent bias.

2.5. Statistical analysis:

All data were analyzed using IBM SPSS Statistics (13). To evaluate the predictive values, the following statistical methods were applied:

Descriptive statistics: Categorical variables were summarized using frequencies and percentages. Continuous variables were summarized using means and standard deviations.

Comparative Analysis: To compare the differences between groups (infected vs. non-infected), Welch's t-test was utilized for continuous data, and (Chi-square X2) tests were used for categorical data (14). To evaluate the level of diagnostic agreement between categorical hs-CRP results (>10 mg/L) and the reference test (UBT), Cohen's kappa (k) coefficient was used.

Diagnostic performance: The analysis in this study focused on determining sensitivity and specificity at the baseline level, in addition to the (PPV) and (NPV) for the three studied tests, (UBT) test, stool antigen (Ag) and hs-CRP.

ROC Curve Analysis: To examine the ability to predict H. pylori infection, ROC analysis was

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performed to accurately determine the (AUC) for the studied tests. This was used to assess the overall diagnostic accuracy and identify optimal cutoff points for the hs-CRP test (15).

Statistical Evaluation of Diagnostic Relationships: Assessing the extent of the relationship between high hs-CRP levels and the presence of H. pylori infection, correlation analyses were performed and confirmed by UBT as a reference test (16). Spearman's rank correlation coefficient (rs) was employed for a non-parametric assessment.

To address the significant imbalance in sample sizes between the groups (n = 147 vs. n = 3) and the differences in variances, both Welch's unequal variances t-test and the non-parametric Mann-Whitney U test were employed to assess the differences in continuous hs-CRP levels between patients who tested positive for UBT and those who tested negative.

Significance level: For all tests included in the study, p-value of less than = 0.05 was considered statistically significant (17).

3. Results:

3.1. Baseline and Demographic Data:

The study included 150 symptomatic patients (89 males, 59.3%, 61 females, 40.7%) with a mean age of 34.19 ± 11.97 years.

The initial screening showed that 98% (n=147) were positive for H. pylori according to the (UBT), while 93.3% (n=140) were positive via the Stool Antigen (Stool Ag) test.

Regarding the indicators of inflammation, the (hs-CRP) levels showed significant variability among the participants, with a minimum value 0.5 mg/L and a maximum value reaching 189.1 mg/L, resulting in an overall mean of 66.19 ±

38.87 mg/L applying the clinical cut-off of >10 mg/L to define positivity: Positive hs-CRP: 143 patients (95.3%), Negative hs-CRP: 7 patients (4.7%).

Welch's t-test showed a highly significant difference in hs-CRP levels between infected and non-infected groups. Patients with a positive UBT had significantly higher hs-CRP levels (67.52 ± 38.11 mg/L) compared to those with a negative result (0.96 ± 0.81 mg/L, $p < 0.001$). Similarly, Stool Ag-positive patients exhibited significantly higher mean hs-CRP levels (88.63 ± 27.67 mg/L) than negative patients (38.42 ± 32.74 mg/L, $p < 0.001$) (Table 1).

Table 1. Diagnostic biomarkers comparing the positive and negative groups, confirmed using the reference standard (UBT).

Diagnostic Test / Variable	Positive Group (n)	Mean ± SD (mg/L)	Negative Group (n)	Mean ± SD (mg/L)	Mean Difference	Welch's t-value	p-value	Cohen's d (95% CI)
UBT Status	147	67.52±38.11	3	0.96±0.81	66.56	20.94	<0.001	1.758 (0.595 – 2.916)
Stool Ag Status	140	88.63±27.67	10	38.42±32.74	50.21	4.67	<0.001	1.731 (0.978 – 2.484)

Notes: UBT = Urea Breath Test; SD = Standard Deviation; CI = Confidence Interval; Ag = Antigen.

The study revealed a very high infection rate, with 147 out of 150 patients (98%) testing positive for *H. pylori* via UBT. It was observed that the average hs-CRP level was higher in the infected group (67.52 ± 38.11 mg/L) compared to that of the non-infected group (0.96 ± 0.81 mg/L), with a highly significant of p-value of <0.001. To confirm a clear relationship between the urea breath test and the C-reactive protein test, Fisher's exact test showed a significant association between UBT status and hs-CRP status ($p < 0.001$). The results showed a highly statistically significant relationship between the pair tests, with a significance level of $p < 0.001$ (Table 2).

Kappa tests (kappa = 0.588) confirmed a moderate agreement between the diagnostic methods.

3.2. Diagnostic Accuracy and ROC Curve Analysis

When using ROC analysis to assess the predictive performance of the C-reactive protein test, the results showed an (AUC) of 1.000 (95% CI: 1.000–1.000, $p < 0.001$). These results indicate that the AUC value for the C-reactive protein test is an ideal indicator for differentiating between *H. pylori* infected and non-infected patients in the study. The results also showed that the cutoff point of 10 mg/L for C-reactive protein provides the higher diagnostic accuracy, with 97% sensitivity & 100% specificity (Chart 1).

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Table 2: Urea breath test results & hs-CRP Cross-tabulation.

UBT Reference Result	hs-CRP (≤10 mg/L) Negative	hs-CRP (>10 mg/L) Positive	Total (N)	Fisher's Exact p-Value
Negative	3	0	3	<0.001
Positive	4	143	147	
Total	7	143	150	

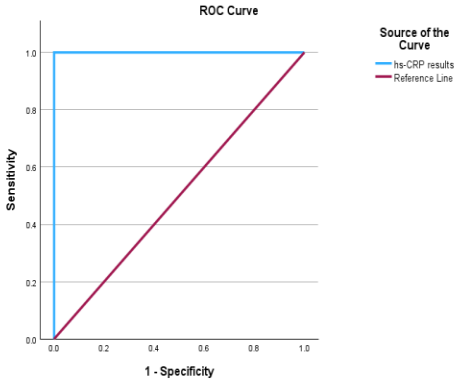


Chart 1. ROC Curve.

3.3. Diagnostic Accuracy Parameters.

In assessing serum hs-CRP using a (cutoff of >10 mg/L) compared to the UBT reference standard, the diagnostic performance metrics were as follows: Sensitivity: 97.28% (95% CI: 93.17% – 99.25%) Specificity: 100.0% (95% CI: 29.24% – 100.0%) Positive Predictive Value (PPV): 100.0% (95% CI: 97.45% – 100.0%) Negative Predictive Value (NPV): 42.86% (95% CI: 15.82% – 74.95%). The low NPV and broad 95% confidence interval can be attributed to the significant imbalance in sample size, as only 3 subjects tested negative for H. pylori according to the reference standard.

A non-parametric correlation analysis indicated a significant positive correlation between quantitative hs-CRP levels and UBT-confirmed H. pylori status ($r_s = 0.620$, $p < 0.001$).

4. Discussion

The primary objective of this study was to evaluate the predictive value of combining the systemic inflammation marker tests, including the UBT and stool antigen test, for the diagnosis of *H. pylori*. The study also aimed to determine whether combining tests provides greater reliability and accuracy in diagnosing the presence and severity of the infection, and reduces the error rate compared to the approach of using a single test (18).

The results showed that the assessment of these non-invasive indicators provides valuable supplementary clinical information. While other traditional methods, such as endoscopy, still yield good results and remain a primary diagnostic option, they are classified as invasive procedures, and the possibility of sampling errors remains, thus limiting diagnostic accuracy and making it dependent on the skills of the endoscopist (19).

This study represents an attempt to explore alternative, accurate, and non-invasive diagnostic tests that provide rapid and precise results regarding the presence or absence of bacteria.

The data in this study showed a very clear difference in levels of hs-CRP between individuals infected with and uninfected by H. pylori. Patients with a positive breath urea test had a relatively high mean hs-CRP level of 67.52 ±

38.11 mg/L, compared to only 0.96 ± 0.81 mg/L in patients with a negative breath urea test ($p < 0.001$). These results support the prevailing view that H. pylori causes inflammation in the digestive tract, leading to elevated hs-CRP levels (20).

The (AUC =1.000) shows that (hs-CRP) gives a very important indicator of infection when a (cut off = >10 mg/L). The (UBT) used as the reference (Gold Standard) in this study, with a high (positivity rate = 98%). Among participants experiencing symptoms of the bacteria, this provides a high and accurate diagnostic capability for infection. Therefore, I emphasize that the results and cutoffs used in this study are specific to high-risk, symptomatic clinical signs and cannot be directly generalized to asymptomatic populations or geographic areas with low prevalence of H. pylori without further studies with multiple geographic centers (21). As for the fecal antigen test (using lateral flow chromatography), it showed a different positivity rate, which was slightly lower at 93.3% (22).

The agreement between the binary hs-CRP classification (>10 mg/L) and the urea breath test (UBT) yielded a Cohen's kappa value of 0.588, indicating moderate agreement. This moderate agreement highlights that, although elevated systemic inflammation often accompanies active infection, individual variations in immune response prevent hs-CRP from replacing the direct detection of antigens or urease activity. (23).

All patients who had taken antibiotics or any type of proton pump inhibitor were excluded because such drugs inhibit the bacterial activity to be detected,

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leading to false-negative results for both the (UBT) and the (stool Ag tests) (19).

The prevalence of H. pylori infection in the studied group may be very high (98%). However, this is a common occurrence among patients with dyspepsia symptoms

One reason for the observed elevated levels of (hs-CRP) in the blood serum of infected patients is the presence of systemic inflammatory biological pathways resulting from the bacterial infection. The bacteria adhere to the wall of the stomach lining and use their virulence factors, which include the cytotoxin A-associated gene (*CagA*) and vacuolar cytotoxin A (*VacA*), to affect the tight cellular bonds, resulting in the stimulation of the human innate immune response, which includes immune cells (phagocytes, neutrophils, and T lymphocytes) and gastric epithelial cells to produce pro-inflammatory cytokines, especially interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-α). In addition to interleukin-6 (IL-6), which plays an important role as a major transporter in the bloodstream, it travels to the liver to stimulate the production of (hs-CRP). As a result, (hs-CRP) is a specific systemic indicator of the extent of inflammation.

The results of this study are consistent with recent studies on *H. pylori* investigating the diagnostic efficacy of non-endoscopic tests and systemic inflammation markers. Systematic studies by authors Best et al. (2018) and Jambi (2022) have shown that non-invasive methods, such as the urea breath test (UBT), Shows a high percentage of sensitivity and specificity exceeding 90–95%. This justifies my choice of UBT as a reliable reference framework in the study communities. (10, 18). Furthermore, a meta-analysis by authors Watanabe and Kotani (2021) revealed a significant decrease in baseline (hs-CRP) after complete treatment of *H. pylori* (6), This evidence confirms our conclusion that the presence of an active infection keeps inflammation levels persistently high

Finally, endoscopy remains the gold standard and primary reference for the assessment and diagnosis of inflammatory conditions, including gastric ulcers, atrophic mucosa, and gastric malignancies such as cancer. It is important to note that non-invasive inflammatory and antigen tests do not replace upper endoscopy; they only provide supplementary value to the initial clinical assessment in uncomplicated primary care settings.

5. Conclusion

In this study, the results showed a significant increase in hs-CRP levels in the serum of patients with confirmed Helicobacter pylori infection using the reference standard (UBT) (25). However, hs-CRP testing cannot replace upper endoscopy for overall structural evaluation, and it should not be used as an independent diagnostic test in itself, but rather as an adjunctive inflammatory marker alongside other established diagnostic tools.

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Ethical Considerations and Compliance with Ethical Guidelines

The study received institutional review board approval (approval no. MU-IRB-0016).

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Conception and design: M.N, A.J, Provision of study: S.H.M, M.H, M.N, A.J, R.K, Collection, and assembly of data: S.H.M, Data analysis and interpretation: S.H.M, M.H, M.N, A.J, Manuscript writing and final approval of manuscript: all authors.

AI Using Declaration

No AI tools were used.

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